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APPLICATION NUMBER: *60/536,766*
FILING DATE: *January 15, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/01393*

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING PROVISIONAL PATENT APPLICATION
Und r 35 USC 111(b)
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PROVISIONAL
APPLICATION

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PROVISIONAL APPLICATION
Under Rule 53(c)

Sir:

Herewith is a PROVISIONAL APPLICATION
Title: ***SYSTEM AND METHOD FOR EXPRESSION PROTEOMICS BASED ON ISOTOPE RATIO MODIFICATION***

Atty. Dkt. PW 81476-307250 (M#) | Whitelegge (Client Ref)

including:
Date: January 15, 2004

1. Specification: 4 pages 1A. ☐ Claim: _____ pages 1B ☐ _____ Abstract pages
2. ☐ Specification in non-English language 3. ☒ Drawings: 1 sheet(s)

4. The invention ☐ was ☒ was not made by, or under a contract with, an agency of the U.S. Government.
If yes, Government agency/contact # = _____

5. ☐ Attached is an assignment and cover sheet. Please return the recorded assignment to the undersigned.

6. Small Entity Status ➡ ☐ is Not claimed ☒ is claimed (**pre**-filing confirmation **required**)
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7. ☐ Attached:

8. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

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(2) Inventor			
	First	Middle Initial	Family Name
Residence			
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9. NOTE: FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet (PAT102A) with sam informati n regarding additional invent rs.

	Large/Small Entity		Fee Code
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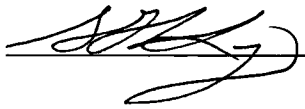
Sig: 

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

JULIAN P. WHITELEGGE

Group Art Unit: Unknown

Appln. No.: Unassigned

Examiner: Unassigned

Filed: January 15, 2004

Atty Dkt: 81476-307250
C# M#

Title: ***SYSTEM AND METHOD FOR EXPRESSION PROTEOMICS BASED ON ISOTOPE RATIO MODIFICATION***

CERTIFICATE OF MAILING VIA U.S. EXPRESS MAIL
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Dated: January 15, 2004

Sherry B. Kolber

APPLICATION FOR A PROVISIONAL
UNITED STATES PATENT
IN THE NAME OF

Julian P. Whitelegge

for

SYSTEM AND METHOD FOR EXPRESSION PROTEOMICS BASED ON ISOTOPE RATIO MODIFICATION

Assigned to:

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SYSTEM AND METHOD FOR EXPRESSION PROTEOMICS BASED ON ISOTOPE RATIO MODIFICATION

FIELD OF THE INVENTION

The invention relates to expression proteomics, and, particularly, to a system and method for analyzing expression proteomics based on a modification or modifications to isotope ratio.

BACKGROUND OF THE INVENTION

There are a number of stable isotope strategies currently used to study biological systems. Each of these strategies is predicated on the use of a complete or near-complete isotope swap in the molecules, sample, or entire organism being analyzed. This process is sometimes referred to as "conversion" with reference to the molecules, sample, or entire organism.

In general, a non-natural isotope is "swapped" for the naturally occurring variety. For instance, ^{15}N may be swapped for ^{14}N, ^{13}C for ^{12}C, or ^{18}O for ^{16}O. A complete (or near-complete) isotope swap may be achieved by any number of strategies well known to those of skill in the art. By way of example, these strategies may include isotope-coded affinity tag ("ICAT") technology, stable isotope labeling with amino acids in cell culture ("SILAC"), enzymatic molecular introduction (*i.e.*, exchange) of ^{18}O, and growth of a biological sample, a cell culture, or an entire organism in stable isotopes. By inducing an isotope swap of this nature, it becomes possible to examine a range of biological processes at the molecular level.

While this technology has important implications and uses in the study of molecular biology, it is significantly limited in its applications. It is generally not applicable, for example, to the study of living humans and other animals. Inducing a complete swap of isotopes in living humans and other animals -- even if it could be achieved, which is by no means a simple assumption -- is likely to present significant health concerns, and to be both tremendously expensive and time-consuming. Thus, in order to implement isotope swapping techniques in the study of such systems, a different methodology is believed to be required. Such a methodology may have a dramatic impact on the study of biology and biological systems, and particularly human and animal biology, as well as other systems that may not be appropriate for study in connection with the conventional methods described above.

DESCRIPTION OF THE INVENTION

The present invention is based on a novel approach to the study of molecular biology, by inducing subtle modifications in the isotopes of target molecules. "Subtle," as used herein with reference to the modification of isotopes included in target molecules, is defined as a "swapping" of, on average, substantially less than all of the isotopes in a target molecule. These subtle modifications may be induced with reference to any suitable target isotope or isotopes, which may include, but are in no way limited to, ^{13}C for ^{12}C, ^{18}O for ^{16}O, and ^{15}N for ^{14}N. Additional isotopes suitable for subtle modification in accordance with alternate embodiments of the present invention will be readily appreciated by those of skill in the art; for instance, deuterium may be swapped for hydrogen.

Subtle alteration of the ratio of ^{13}C to ^{12}C may be particularly advantageous in connection with the methods of the present invention, insofar as these methods are implemented in connection with proteomics. Carbon is the most abundant constituent of proteins, and, thus, a small change in the ratio of ^{13}C to ^{12}C has the most dramatic effect upon isotopic distribution; changing this ratio from 100:1 to 100:2 (or 200:1), for instance, may have quite a dramatic effect. In the context of proteomics, this subtle alteration of isotopic ratio can be measured from the isotopic distribution of peptide ions; thereby providing a means of stable isotope tagging that does not require full conversion to a non-natural isotope. For example, Fig. 1 illustrates a MALDI readout from Synechocystis cultures in which subtle modifications of target molecules were induced (normal IR, +1.5%, +3.0% and +6% ^{13}C, respectively; from top to bottom of Figure), in accordance with an embodiment of the present invention.

The methods of the present invention are by no means limited to the study of proteomics, however. In fact, the invention may find application in a host of biological systems, as well as non-biological systems (*i.e.*, in the study of any system in which isotopes may be subtly modified and thereafter analyzed by the methods described herein).

Methods for inducing the subtle modifications incorporated in various aspects of the present invention will similarly be recognized and may be readily implemented by those of skill in the art without undue experimentation. In addition to the use of variants of the technology described above (*i.e.*, ICAT, SILAC, enzymatic exchange, and growth in stable isotopes), other means for inducing the subtle modification of isotopes can be readily ascertained. For example, a living animal may be fed a diet that includes the non-natural isotope or isotopes sought to be

introduced into their internal physiology; diet influences actual isotope ratios in animals, including humans. Such a diet may include, for example, food (*e.g.*, animal chow) supplemented with the non-natural isotope, or, in another embodiment of the invention, the diet may include deuterated or deuterium-enriched water. Alternatively, the isotope may be introduced by pharmacological means (*e.g.*, a diet supplement), or by other conventional forms of administration (*e.g.*, injection of saline consisting of the non-natural isotope).

Once the target molecule (or molecules) has been subtly modified, it may be studied by a number of different technologies. For example, isotope ratio mass spectrometry ("IRMS") may be employed, whereby levels of different isotopes (*e.g.*, ^{13}C and ^{12}C) may be measured after conversion to carbon dioxide (*e.g.*, by combustion). Alternatively, the isotope ratio may be measured by calculation from the peptide mass spectra obtained by various forms of mass spectrometry (*e.g.*, MS-MS). Specific protein molecules can be identified by MS-MS in connection with an appropriate database search, or isotopic distribution can be estimated using averagine (*i.e.*, a model amino acid with elemental components occurring at frequencies deduced from the PIR database). In particular embodiments of the invention, high resolution mass spectrometry, such as Fourier transform mass spectrometry ("FTMS"), may be particularly advantageous and accurate. Moreover, in instances where peptide sequences (and, thus, elemental compositions) are known, the measurements attainable on high-resolution instrumentation, such as mass spectrometers employing FTMS, may be highly accurate.

The present invention has a range of applications. In one embodiment, the invention may be used in connection with isotope coding by subtle alteration of isotope ratio in proteins. This may be particularly useful in connection with proteomics. For instance, one may study the relative expression of various proteins in a biological system. Two (or more) samples can be distinguished by their isotope ratios; thereby allowing mixing and relative expression measurement by comparison of peak height/areas (*i.e.*, in a MALDI readout). The isotope ratio of a peptide in a mixture is determined by relative contribution from non-labeled as compared with labeled material.

In another embodiment, the present invention may be used to study protein turnover (*e.g.*, one may monitor metabolic or transcriptional activity by seeking out newly altered or transcribed proteins, respectively). Protein turnover may be measured using pulse-phase protocols. For instance, if a human begins to eat food with an altered isotope ratio, then this will first be

observed in rapidly turning over proteins. Slow turning over proteins will be last to manifest the change in isotope ratio.

Among the benefits of employing the methods of the present invention in connection with, for example, proteomics, is the significant cost savings and the ability to study systems that were heretofore impossible to study by way of isotope swapping.

While the description above refers to particular embodiments of the present invention, it will be understood that many modifications may be made without departing from the spirit thereof. Such alternate methodologies and procedures may be readily implemented without undue experimentation. The presently disclosed embodiments are therefore to be considered in all respects as illustrative and not restrictive.

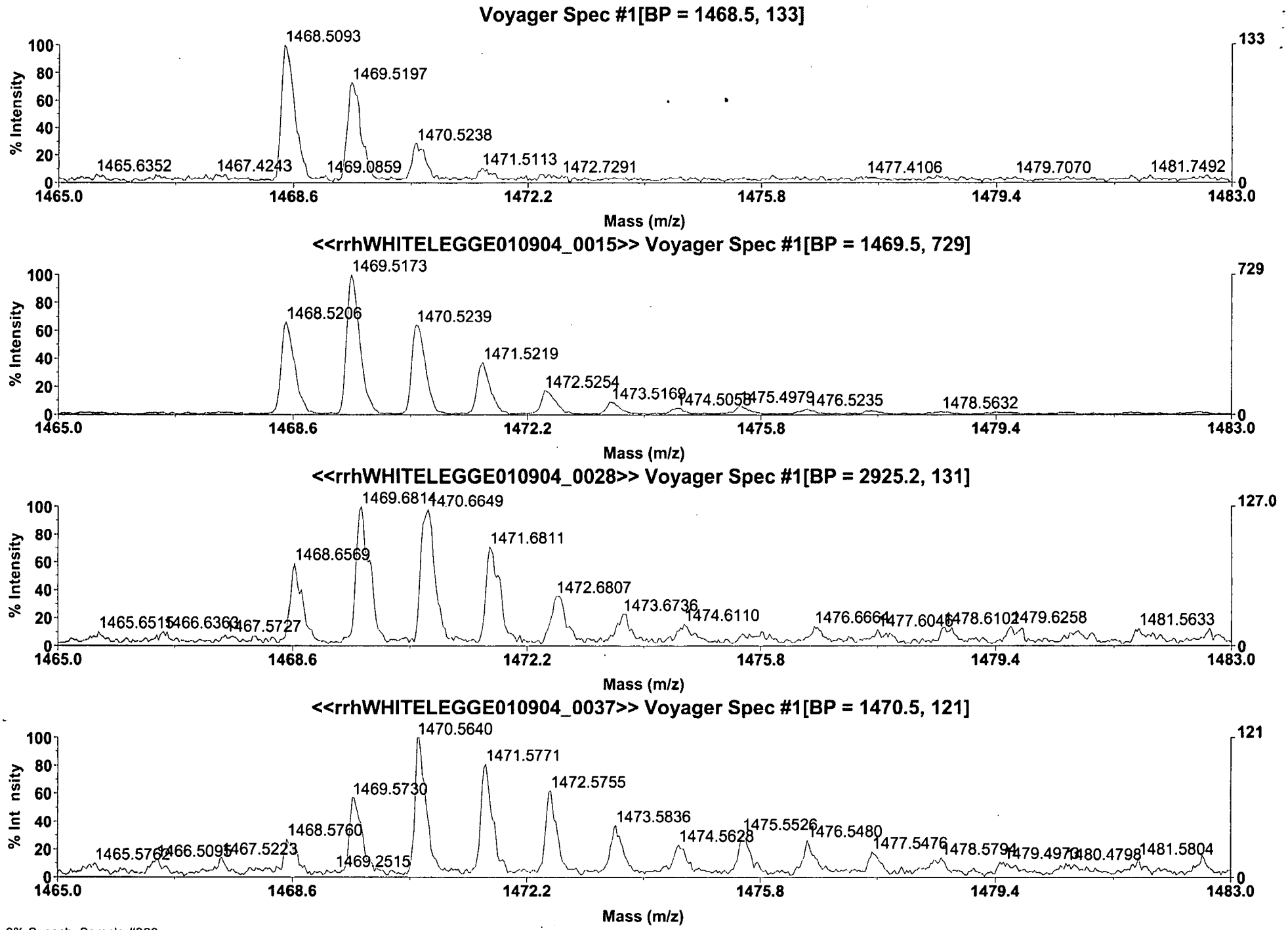

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FIGURE 1